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THE SURVIVAL OF *VAUCHERIA* (VAUCHERIACEAE) PROPAGULES IN NEW ENGLAND RIPARIAN SEDIMENTS AFTER REPEATED FREEZE/THAW CYCLES

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ABSTRACT. Previous studies have demonstrated the ability of the alga *Vaucheria* to survive prolonged and stressful periods of desiccation and freezing. However, even during harsh New England winters, the top few centimeters of floodplain alluvium or stream bank mud, where *Vaucheria* is often found, experience repeated thawing and refreezing events. Muds from two Connecticut riparian sites known to contain propagules from as many as eight species of *Vaucheria* were collected in spring and summer, then subjected to a variety of freeze/thaw (F/T) cycles. Six species of *Vaucheria*—*V. aversa*, *V. frigida*, *V. geminata*, *V. prona*, *V. taylorii*, and *V. undulata*—have demonstrated survival tolerance to multiple F/T cycles of intervals from 1–10 days.

Key Words: freeze/thaw cycles, propagules, riparian sediments, seed banks, *Vaucheria*

In New England winters, the natural deep-freezing of subsurface soil for up to four months is common. However, the upper surface of moist stream and riverbank alluvium above the water line rarely remains frozen for extended periods of time. On sunny winter days above 0°C, the top few centimeters of mud will thaw, only to refreeze at night. At times, above-freezing winter temperatures are maintained long enough to thaw the mud for several days before refreezing. Organisms that live in the top few centimeters of mud are subject to these freeze/thaw (F/T) events and therefore must withstand such conditions to survive.

The freshwater members of the cosmopolitan yellow-green alga *Vaucheria* (Vaucheriaceae, Tribophyceae, Chrysophyta) are commonly encountered living in and on riparian muds (Schneider et al. 1999). As the coenocytic siphons of *Vaucheria* grow, they weave in and out of their mud substrate and often between the siphons of sympatric species, forming what is referred to as a "felt-like turf." In order to survive the environmental extremes such as desiccation and freezing that impinge upon it, this alga, like so many others in their specific environments, has developed propagules as resting structures that are deposited in the floodplain soils or riverbank muds. When environmental conditions are optimal, *Vaucheria* grows and reproduces forming zygotes (oospores). But when conditions are limited by abiotic components in the environment, the siphons of *Vaucheria* can form sporangia or "cyst-like" resting fragments (Dunphy et al. 2001). We have recently shown that the propagules of eight species—*V. aversa* Hassall, *V. bursata* (O. F. Müll.) C. Agardh, *V. frigida* (Roth) C. Agardh, *V. geminata* (Vaucher) Alph. de Candolle, *V. prona* T. A. Chr., *V. taylorii* Blum, *V. uncinata* Kütz., and *V. undulata* C. C. Jao—were able to survive desiccation in the laboratory from 63–383 days (Dunphy et al. 2001). The survival of each of these species was likely due to the deposition of a "seed bank" of propagules left in the mud during or after periods of active growth, and the physiological tolerance of these resting cells to prolonged periods without soil moisture. Since the freeze/thaw phenomenon is so prevalent in Connecticut riparian muds and such an important physiological stress for *Vaucheria*, this study examines the survival tolerance of the propagules of these species to repeated freezing and thawing for varying numbers of cycles.

STUDY AREAS

The two Connecticut collection sites where we have observed "felt-like" turfs of *Vaucheria* with high levels of species sympatry (Dunphy et al. 2001; Schneider et al. 1999) were selected for this study:

1. Nipmuck Trail (NK)—Ashford, Windham County, approx. 3 km from an entrance to the Nipmuck Trail, a portion of the Mohawk Trail system, on Conn. Rt. 74 [41°51.301′N, 72°12.821′W (Garmin™ GPS 12, v. 4.57)];
2. Scantic River (SCR)—Enfield, Tolland County, floodplain directly beneath the bridge on Conn. Rt. 190 that crosses over the Scantic River near the intersection with Conn. Rt. 191 (41°58.966′N, 72°30.969′W).

Eight species of *Vaucheria* have been found at SCR—*V. av-*

ersa, *V. bursata*, *V. frigida*, *V. geminata*, *V. prona*, *V. taylorii*, *V. uncinata*, and *V. undulata*—while all of the above except *V. taylorii* are known from NK (Dunphy et al. 2001).

MATERIALS AND METHODS

Bulk samples of mud containing propagules from each site were collected on 24.iv.2000, 5.vii.2000, and 23.viii.2000. The procedures for field collection and preparation of a homogenous mud in the lab are described in Dunphy et al. (2001). After being left uncovered for five days, the moist mud slurry was cut into blocks approximately 36 cm^2, placed in zippered plastic bags and frozen to 0°C. Control mud samples were immediately placed in individual plastic culture dishes (2.3 cm × 8.5 cm) with Bold's basal medium (Bischoff and Bold 1963), and cultured as in Dunphy et al. (2001).

Bags of mud totaling 33 blocks per collection site and date were removed from the freezer for each F/T experiment. The bags were placed in a growth chamber set at 15°C, and the mud was allowed to thaw for the specified length of time for each experiment. Thawing periods of 1–5, 7, and 10 days were used. Three blocks from each collection site were then removed from their bags and placed in individual culture dishes with 4–5 ml of culture medium, labeled, and placed back in the growth chamber. The bags containing the remaining blocks were returned to the freezer for 2 days at which point the cycle was repeated until all of the mud blocks were cultured.

Cultures were monitored for signs of *Vaucheria* siphons using light microscopy. Vouchers of reproductive materials from numerous samples were prepared (20% or 40% Karo™ corn syrup, 1% aqueous aniline blue, and 1 N HCL in a ratio of 20:1:1) and deposited in Herbarium C. W. Schneider at Trinity College, Hartford.

RESULTS

Because the three replicate control dishes of mud collected from each site in April, July, and August produced similar species, survival data for NK and SCR were combined in Table 1. In the control dishes for NK, four species developed: *Vaucheria aversa*, *V. geminata*, *V. prona*, and *V. undulata*. In the controls for SCR, we discovered *V. frigida*, *V. prona*, and *V. undulata*

Table 1. Freeze/thaw cycle survival of six *Vaucheria* species from two Connecticut riparian sites (NK, SCR), and percent survival in total experimental dishes. Numbers represent the maximum number of F/T cycles a species survived at each site for specified number of thaw days per cycle. Species appearing in control dishes are denoted by asterisks (*). Culture dishes that never produced gametangia, hence remaining unidentified, are noted as *V.* spp.

Species	Collection Site	No. F/T Cycles Survived at Various No. Thaw Days/Cycle 1	2	3	4	5	7	10	Species Occurrence (% of Total Dishes)
V. aversa	NK*	–	–	8	4	5	7	4	24.4
	SCR	1	–	–	4	4	–	1	0.05
V. frigida	NK	–	–	–	–	–	–	–	0.00
	SCR*	–	7	6	–	8	–	–	0.03
V. geminata	NK*	–	–	–	7	2	–	–	0.01
	SCR	–	7	–	–	–	3	2	0.02
V. prona	NK*	–	5	8	–	–	2	4	0.07
	SCR*	9	8	6	4	7	6	5	26.7
V. taylorii	NK	–	–	–	–	–	–	1	0.01
	SCR	–	–	–	–	–	–	1	0.01
V. undulata	NK*	–	5	–	1	–	–	2	0.04
	SCR*	2	7	6	4	–	5	5	0.07
V. spp.	NK	10	9	–	5	7	6	1	12.2
	SCR	3	9	8	6	9	–	6	15.4

(Table 1). Several species appeared in experimental dishes from the two sites despite not appearing in control cultures; in fact, only *V. prona* and *V. undulata* were found in the control dishes from both sites. *Vaucheria taylorii* was found in neither control, yet appeared in one experimental dish from each site (Table 1). This represents the first report of *V. taylorii* from NK, thus the same eight species are known from both collection sites. Problems associated with assessing muds containing unknown quantities of propagules in the "seed banks" are discussed by Dunphy et al. (2001), but the low frequency of appearances of certain species in experimental dishes and the lack of the same species in the controls suggest small quantities of propagules in our collected muds.

Each of the species found in the control dishes was able to survive multiple cycles of freezing, followed by one or more thaw days in at least some of the experimental dishes (Table 1). In some instances, the survival of a species after experimental treatment may in fact result from a single reproductive population in one of the three replicate dishes for each site. Combined, 45% of the 375 experimental dishes developed *Vaucheria*—42% of those from NK and 49% from SCR. However, in many of the experimental dishes, *Vaucheria* siphons never became reproductive even after months in culture, thus disallowing species identifications in 12% of NK dishes and 15% of those from SCR. Only two species were found in greater than 1% of all of the experimental dishes from a site: *V. aversa* in 24% from NK, and *V. prona* in 27% from SCR. *Vaucheria aversa* appeared more frequently in culture dishes in the late winter to early spring regardless of collection time or experimental regimen, similar to findings for rehydrated desiccated muds containing *V. aversa* propagules from a previous study (Dunphy et al. 2001). The other four species were found at a much reduced frequency (Table 1). As noted above, *V. taylorii* appeared in only two cultures, one from SCR and the other from NK, the latter representing the first report from this site.

Three species survived in most of the experimental treatments of 1–10 days thawing after 2 days of refreezing; *Vaucheria aversa* from NK, and *V. prona* and *V. undulata* from SCR (Table 1). *Vaucheria prona* was found growing in SCR dishes after 9 cycles with 1 day of thaw. For this species at SCR (the site and species that provided the greatest amount of data), as the number of thaw days increased in the trials, we observed that *V. prona* survived the greatest number of F/T cycles with the shortest thaw period (1 day; Table 1). The number of F/T cycles that *V. aversa* and *V. undulata* survived compared with the number of days thawed shows no obvious trend. The remaining species and sites had less complete survival data, no doubt due to their lesser presence in the "seed bank." Nevertheless, all six *Vaucheria* species showed tolerance to F/T stresses more extreme than we suspect they are exposed to at the sites from which they were collected, including the species with a lesser presence. *Vaucheria frigida* survived 8 cycles with 5 days of thaw (SCR), while *V. taylorii* (NK, SCR) and *V. geminata* (SCR) survived 1 and 2 cyles, respectively, with 10 days of thaw.

DISCUSSION

Few studies have been made on the effects of freezing and thawing on algae, although several have looked at the survival of bacteria, often in boreal and arctic soils. Skogland et al. (1988) discovered that a single F/T cycle could kill as many as 50% of a viable soil microbial population, and Schimel and Clein (1996) noted that following these environmental events, the dead cells contributed significant nutrients to the soil for surviving organisms. Other studies have focused on morphological or molecular and biochemical responses of cells disrupted by freezing and thawing, from bacteria and fungi to cereal crop protoplasts (Morris et al. 1988; Steponkus et al. 1983). Although many studies have looked at the effects of prolonged freezing and cryopreservation in unicellular and filamentous algae (Ginsburger-Vogel et al. 1992; Morris 1978), little is known about their survivability after repeated cycles of freezing and thawing, conditions many stream and riparian algae are exposed to in their native environments. In one study, Hawes (1990) observed that the vegetative cells of a filamentous green alga from Antarctic streams—an unidentified species of *Zygnema*—could survive the repeated freezing and thawing cycles typical of austral summers with little effect, but that prolonged exposure to −20°C winter temperatures caused extensive cell mortality. He concluded that the few winter-surviving cells in filaments became the "seed" population for summer growth in *Zygnema* without the involvement of resting spores or other specialized structures typically utilized by freshwater filamentous green algae to survive stressful environmental events (Coleman 1983).

An organism such as *Vaucheria*, whose propagules can survive over a year in desiccated mud, would be expected to survive other environmental stresses normally encountered in its habitat, such as winter freezing in New England. We have observed that *Vaucheria* propagules, including all of the species tested herein for survival in repeated F/T cycles, survive in moist, freezing mud for over a year (unpubl. data). In the present study, the propagules of six species of *Vaucheria* have been shown to survive the stress of multiple F/T cycles (Table 1), conditions these species might normally encounter in a typical Connecticut winter in the upper soil strata of riparian habitats. *Vaucheria aversa*, *V. prona*, and *V. undulata* were the most frequently encountered species in our experimental dishes, showing the greatest survival after repeated F/T cycles. Presumably, these species had left the greatest numbers of propagules in the "seed bank" in our collected muds. Despite being only sporadically found in our culture dishes, the remaining three species, *V. frigida*, *V. geminata*, and *V. taylorii*, nevertheless survived experimental treatments in some of the dishes, showing their ability to survive repeated F/T cycles. Although *V. bursata* and *V. uncinata* were previously reported from both the NK and SCR sites (Dunphy et al. 2001), neither appeared in any of the control or experimental cultures. It is therefore reasonable to assume that their propagules were not present in the mud collections made for this study.

In this and past studies, we have seen *Vaucheria* siphons appear above the substrate surface in as little as ten days after thawing frozen muds. Propagule germination must therefore occur much earlier, within the first few days post thaw. The refreezing of muds that have been thawed for prolonged periods of time tests not only the ability of propagules to survive refreezing, but the ability of germinated siphons to survive as well. With ten-day thaw intervals, a large percentage of propagules will likely germinate and thus become susceptible to freezing injury with each F/T cycle. Without the cellular partitioning found in filamentous freshwater algae such as the chlorophyte *Zygnema* (Hawes 1990), the siphons of *Vaucheria* would appear to have fewer options for cellular protection and therefore be more susceptible to mortality. If some percentage of the "seed bank" survives after each F/T cycle, the species with the most numerous propagules should show the greatest success even if its ability to withstand the stress is no greater than any other species. Therefore, it would appear to be important for species to deposit a large number of propagules in the environment to have a greater chance of surviving F/T stress, as it appears that individual mortality must take its toll. Because *V. aversa*, *V. prona*, and *V. undulata* were commonly collected species in a great sampling of riparian Connecticut habitats (Schneider et al. 1999), and therefore could have deposited the most numerous propagules in our NK and SCR samples, it is not surprising that they showed greater success with longer thawing times than the other species (5 cycles with 10-day thaw intervals). Thus, they continue to appear to be ecologically opportunistic, having already demonstrated survivability after long periods of desiccation—145, 359, and 383 days, respectively (Dunphy et al. 2001).

Vaucheria prona and *V. undulata* survived the greatest number of F/T cycles (5) with thaw periods of 10 days. In Connecticut, the surfaces of floodplain alluvium or river banks would rarely, if ever, thaw once for ten continuous days and then refreeze during the winter freezing period of December to March. These two species, along with *V. aversa* and *V. geminata*, have shown great success in surviving multiple F/T cycles with longer thaw intervals. If they can survive such an extreme and repeated stress, unlikely to occur in their natural habitats, it seems probable they can survive any series of F/T cycles that would naturally occur in New England, assuming the propagules have not all germinated and died in the young siphonous form. The survival of all six *Vaucheria* species exposed to the stress of repeated freezing and thawing cycles further demonstrates the ability of this alga to survive severe environmental stress. Survival appears to depend not only on the species' ability to physiologically handle the stress of repeated F/T cycles and the length of thaw intervals, but also upon the abundance of their propagules in a given habitat. Long thaw intervals more than likely allow the germination of *Vaucheria* propagules, and it would appear young siphons would be more vulnerable to repetitive refreezing than resting propagules left ungerminated.

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